DATA EVALUATION RECORD

1. **CHEMICAL**: NTN 33893
   Shaughnessy No. ~~129059~~ 129099

2. **TEST MATERIAL**: Technical NTN 33893, 95.4%.

3. **STUDY TYPE**: Acute Toxicity Test for Freshwater Invertebrates, Daphnia magna.

4. **CITATION**: Young, B. and S. Hicks. 1990. "Acute Toxicity of NTN 33893 to Daphnia magna". Analytical Bio-Chemistry Laboratories, Inc.. P.O. Box 1097, Columbia, Missouri 65205. Laboratory Report No. 37862. Submitted by Mobay Corporation, Agricultural Chemicals Division, P.O. Box 4913, Kansas City Missouri 64120. US EPA MRID No. 420553-17.

5. **REVIEWED BY**:

   Dana Lateulere, Biologist
   Ecological Effects Branch
   Environmental Fate and
   Effects Division

   Signature: Dana Lateulere
   Date: 3/27/92

6. **APPROVED BY**:

   Ann Stavola, Section Head, 5
   Ecological Effects Branch
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   Effects Division

   Signature: Ann Stavola
   Date: 3/27/92

7. **CONCLUSIONS**: This study is scientifically sound and fulfills the guideline requirments. The 48 hour EC50 as determined by the Binomial method using mean measured concentrations is 85.2 mg/L. The LOEC is 71 mg/L based on observations of abnormal behavior. The NOEC is 42 mg/L. Based on the determined EC50, NTN 33893 technical is classified as slightly toxic to Daphnia magna.

8. **RECOMMENDATIONS**:

9. **BACKGROUND**: This study was submitted as part of registration and EUP requirements.

10. **DISCUSSION OF INDIVIDUAL TESTS**: N/A.

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11. MATERIALS AND METHODS:

A. Test Animals: The Daphnia magna used in the test were cultured at the ABC facilities in hard blended water prepared to a total hardness of 160-180 mg/L (as $CaCO_3$). The adult daphnids were fed a suspension of algae species supplemented with trout chow and yeast. The daphnids were fed this at least every three days. The original culture of daphnids at ABC Labs were identified to species using the taxonomic key presented by Pennak. A total of 120 instar were used for the definitive bioassay.

B. Test System: The static bioassay was conducted in 250-ml glass beakers containing 200 ml of daphnid culture/test water. The depth and diameter of the test vessels were approximately 9 and 6.5 cm. All test vessels were covered with loose-fitting petri dish covers to minimize evaporation and prevent contamination during the study. These vessels were kept at 20 (+/- 1) ^{o}C in a temperature controlled water bath with a continuous recording of the water bath temperature maintained during the study. The lighting was maintained at 50-70 footcandles on a 16 hour daylight photoperiod with 30 minute simulated dawn and dusk periods.

C. Dosage: Nominal concentrations tested were: 0, 16, 27, 45, 75 and 125 mg/L. The mean measured test concentrations: 0, 15, 25, 42, 71 and 113 mg/L.

D. Design: The definitive study was initiated using five concentrations in duplicate of the test compound and ABC control with ten daphnid instars. Daphnids were impartially placed into test vessels within 30 minutes of test solution preparation. All concentrations were observed once at 4, 24, and 48 hours for immobility and abnormal effects. Observations of abnormal effects included daphnid laying on bottom, daphnid coated with extraneous material and daphnids antennae coated with extraneous material. Water chemistry parameters of temperature, dissolved oxygen and pH were performed on all test concentrations in replicate "A" at 0-hour and in replicate "B" at 48 hours.

E. **Statistics:** Statistical analysis of the concentrations vs. effect data was employed by a computerized program that calculated the EC50 and its 95% confidence interval using the binomial, moving average and the probit method. The method selected for presentation is the one which gives the narrowest confidence limits.

12. **REPORTED RESULTS:** The measured test concentration yielded an average of 94% of nominals for the 0-hour sampling. By 48 hours they had decreased to 91% of the nominals. The dissolved oxygen concentrations ranged from 8.0 to 8.4 mg/L. These values represented 94 and 97 percent saturation at 21°C and were considered adequate for testing. The pH values of the test solutions ranged from 8.3 to 8.4. The temperature in the "A" replicates was 21°C for the duration of the test; the temperature in replicate "B" was 20°C for the duration of the test.

The 48 hour EC50 was determined to be 85 mg/L with 95% confidence interval of 71-113 mg/L. The no-effect concentration was 42 mg/L. The slope of the dose response curve was 11.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The 48 hour static EC50 of NTN 33893 technical to Daphnia magna is 85 mg/l with 95% confidence interval of 71 - 113 mg/L. The NOEC observed was 42 mg/L after 48 hours, which was based on the absence of immobility and other abnormal effects at this level.

Quality Assurance Inspection was conducted for compliance verification by the Quality Assurance Unit. It was also stated that this study was conducted in compliance with the Good Laboratory Practice Standards, 40 CFR Part 160.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. **Test Procedure:** The test procedures were in accordance with Subdivision E, and SEP guidelines..

B. **Statistical Analysis:** Toxanol was used to determine the LC50 and the 95% confidence interval.

C. **Discussion/Results:** The 48 hour EC50 as determined by the Binomial method using mean measured concentrations is 85.2 mg/L. The LOEC is 71 mg/L based on observations of abnormal behavior. The NOEC is 42 mg/L. Based on

the determined EC50, NTN 33893 technical is classified as slightly toxic to Daphnia magna.

D. **Adequacy of the Study:**

(1) Classification: Core.

(2) Rationale:

(3) Repairability:

Table 5

Individual Immobility and Behavioral Observations During the Acute Toxicity Test of NTN-33893 to Daphnia magna

Mean Measured Concentration mg/L	Rep.	Number Placed in Test Vessel	Cum. Immob.	a Obs.	Cum. Immob.	a Obs.	Cum. Immob.	a Obs.
Control	A	10	0	--	0	--	0	--
	B	10	0	--	0	--	0	--
15	A	10	0	--	0	--	0	--
	B	10	0	--	0	--	0	--
25	A	10	0	--	0	--	0	--
	B	10	0	--	0	--	0	--
42	A	10	0	--	0	--	0	--
	B	10	0	--	0	--	0	--
71	A	10	0	--	0	--	2	2OB,CT 6N
	B	10	0	--	0	--	0	3CT,7N
113	A	10	0	--	0	--	10[b]	--
	B	10	0	--	0	--	10[b]	--

[a] Unless otherwise indicated, the test water was clear and free of precipitate and daphnids were normal in appearance and behavior. The following abbreviations were used for observations: OB = On Bottom; CT = Coated with Extraneous Material; N = Normal

[b] Daphnids coated with extraneous material on antennae

Table 6

Water Quality Measurements During the Acute Toxicity Test of NTN-33893 to Daphnia magna

Mean Measured Concentration (mg/L)	Water Quality					
	Replicate A			Replicate B		
	0-Hours			48-Hours		
	Temp.[a] °C	D.O.[b] mg/L	pH[c]	Temp.[a] °C	D.O.[b] mg/L	pH[c]
Control	21	8.1	8.3	20	8.3	8.3
15	21	8.2	8.3	20	8.4	8.3
25	21	8.2	8.3	20	8.2	8.3
42	21	8.1	8.3	20	8.1	8.3
71	21	8.1	8.3	20	8.2	8.3
113	21	8.0	8.3	20	8.3	8.4

[a]Temperature - digital thermometer.

[b]Dissolved oxygen concentrations - YSI 54 ARC Dissolved Oxygen Probe.

[c]pH - Corning 140 pH/mV meter with Sensorex Model S200C electrode.

Note: Dissolved oxygen saturation at the test temperatures of 20°C and 21°C are 8.7 and 8.5 mg/L (corrected for altitudinal pressure at ABC Labs), respectively.

LATEULERE NTN 33893 TECH DAPHNID EC50

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
113	20	20	100	9.536742E-05
71	20	2	10	2.012253E-02
42	20	0	0	9.536742E-05
25	20	0	0	9.536742E-05
15	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 71 AND 113 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 85.17101

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE
PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE
NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

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